SOLID MICROPARTICLES FOR GENE DELIVERY

This application is a continuation of application Ser. No. 08/265,966, filed Jun. 27, 1994, now abandoned.

BACKGROUND OF THE INVENTION

A variety of techniques have been used to introduce foreign genes into cells. Physical methods include co-precipitation with calcium phosphate, electroporation, and particle bombardment. While these direct transfer techniques are adequate in vitro, they are impractical in vivo. Promising in vivo gene therapy relies on a carrier such as viral vectors or liposomes for delivery. There are still lingering safety concerns for viral vectors. Another limitation is the size of the DNA sequences, usually limited to 7–8 kb, that can be incorporated into the viral vector. Liposomes, on the other hand, have low loading level in general. In both cases, there is the issue of cell or tissue specificity for these gene delivery systems.

Controlled drug delivery has significantly improved the success of many drug therapies (Langer, R., 1990, New methods of drug delivery, *Science,* 249:1527–33; Poznansky, et al., 1984, Biological approaches to the controlled delivery of drugs: a critical review, *Pharmacol. Rev.*, 36:277–336). A major goal of drug delivery is to localize the drug to the target site. These targeted delivery systems often take the form of injectables composed of liposomes (Gregoriadis, G., 1988, Liposomes as Drug Carriers, New York: Wiley; Litzinger, et al., 1992, Phosphatidylethanolamine liposomes: drug delivery, gene transfer and immunodiagnostic applications, *Biochimica et Biophysica Acta.*, 1113:201–27) and microspheres made of proteins (Cummings, et al., 1991, Covalent coupling of doxorubicin in protein microspheres is a major determinant of tumor drug deposition, *Biochem. Pharm.*, 41:1849–54; Verrijik, et al., 1991, Polymer-coated albumin microspheres as carriers for intravascular tumor targeting of cisplatin, *Cancer Chemother. and Pharm.*, 29:117–21; Tabata, et al., 1988, Potentiation of antitumor activity of macrophages by recombinant interferon alpha A/D contained in gelatin microspheres, *Jpn. J. Cancer Res.*, 79:636–646), polysaccharides (Rongved, et al., 1991, Crossed-linked, degradable starch microspheres as carriers of paramagnetic resonance imaging: synthesis, degradation, and relaxation properties, *Carbohydrate Res.*, 145:83–92; Carter, et al., 1991, The combination of degradable starch microspheres and angiotensin II in the manipulation of drug delivery in an animal model of colorectal metastasis, *British J. Cancer*, 65:37–9), and synthetic polymers (Davis, et al., 1984, Microspheres and Drug Therapy, *Amsterdam*; Eldridge, et al., 1991, Biodegradable microspheres as a vaccine delivery system, *Molec. Immunology*, 28:287–94; Pappo, et al., 1991, Monoclonal antibody-directed targeting of fluorescent polystyrene microspheres to Peyer's patch M cells, *Immunology*, 73:277–80). Polymeric systems share some of the advantages of liposomal systems such as altered pharmacokinetics and biodistribution. While liposomes might have better prospects of biocompatibility and potential for fusion with cells, polymeric microspheres have more controllable release kinetics, better stability in storage, and higher drug-loading levels for some classes of compounds.

We have previously synthesized microspheres by the complex coacervation of gelatin and chondroitin sulfate (Truong, et al., 1993, A target-specific microspheres drug delivery system made of enzymatically degradable gelatin and chondroitin sulfate coacervates, *Controlled Release Society*, Abstract #1336; Azhari, et al., 1991, Protein release from enzymatically degradable chondroitin sulfate/gelatin microspheres, *Intern. Symp. Control. Rel. Bioact. Mater.*, 18). These microspheres could be stabilized by cross-linking with glutaraldehyde, the extent of which controls the degradation and drug release rate. Biodegradability of these microspheres in serum is effected by presence of metalloproteinases such as gelatinase, collagenase, and trypsin.

Thus there is a need in the art for a targeted DNA delivery system which can provide controlled release, is simple to make, is stable, is cost effective, has a high DNA loading level, and is relatively non-immunogenic.

SUMMARY OF THE INVENTION

It is an object of the invention to provide polymeric particles for delivery of DNA to cells.

It is an object of the invention to provide a method of making polymeric particles for delivery of DNA to cells.

It is another object of the invention to provide a method of delivering DNA to cells using polymeric particles.

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment a microparticle for gene delivery to specific targets is provided. The microparticle comprises gelatin and DNA, and a linking molecule or a targeting ligand is attached to the surface of said microparticle.

In another embodiment of the invention a method of forming microparticles for gene delivery to specific targets is provided. The method comprises the steps of: forming microparticles by coacervation of DNA and gelatin; and adhering a linking molecule or a targeting ligand to the surface of the microparticles.

In yet another embodiment of the invention a method for introducing genes into cells is provided. The method comprises incubating cells to be transfected with solid microparticles comprising gelatin and DNA, wherein a targeting ligand is attached to said microparticle's surface, said targeting ligand binding to the surface of said cells.

Thus the present invention provides the art with an attractive DNA delivery system which is simple to prepare, is cost effective, has controlled release ability, is storage stable, and is biocompatible.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**. Schematic diagram showing the synthesis of gelatin-DNA coacervates.

FIG. **2**. Gel electrophoresis of cDNA before and after encapsulation. (std=standard; Sup=supernatant, Pellet= microparticles pelleted by centrifugation).

FIGS. **3**(A–B). Controlled release of intact LAMP-1 cDNA was demonstrated in vitro. The microparticles were cross-linked with glutaraldehyde at various glutaraldehyde concentrations then degraded with trypsin.

FIG. **3**A shows the time course of DNA release at various glutaraldehyde-cros-linking levels.

FIG. **3**B shows (on gels and densitometer tracing) the DNA which was released from the microparticles at various times and at various levels of glutaraldehyde-cross-linking.

FIGS. **4**(A–D). Fluorescent images of U937 cells transfected by controls and LAMP-1 cDNA-loaded microparticles (at day 3 post-transfection).

FIG. **4**A: anti-DC44 microparticles without cDNA;

FIG. **4**B: calcium phosphate transfection;